Rice Leaf Extract for Kidney Damage Prevention in Plumbum-Exposed Rats

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Abstract. Plumbum (Pb) is a heavy metal affecting renal tubular epithelial cells and causing necrosis. This study aimed to demonstrate the effectiveness of rice leaf extract in degeneration and necrosis prevention of renal tubule epithelium exposed to lead. Twenty eight rats were divided into a control and 3 treatment groups all exposed to Pb by 0.5 g/ kg/ day, but only the treatment groups had received rice leaf extract in varied doses for 8 weeks. Results from Kruskal-Wallis and sphearman rho tests showed that the number of normal cells, degeneration and necrosis were significantly different (0.03; 0.05; 0.04) between control and treatment groups with a strong positive correlation of treatment (r = 0.97) in normal cells and a strong negative one in cell degeneration and necrosis. (R = -0.92 and r = -0.93). As conclusion, rice leaf extract showed ability in the prevention of kidney damage in rats exposed to lead.

INTRODUCTION

One of the most hazardous air pollutants is Plumbum (Pb) or lead, which could cause health problems and threaten life. The lead poisoning could be sourced from vegetables, batteries, paints, cosmetics, jewelry, children's toys, gasoline, etc. Several large cities, such as Jakarta, Bandung, Semarang, Surabaya, Medan, and several other cities have significant potential for the occurrence of Pb poisoning (Suherni, 2010). Health problems that could arise by this condition is the occurrence of degeneration and necrosis of renal tubular epithelial cells.

Toxication caused by Pb in the body affects various tissues and organs. The organs in the body being targeted by Pb toxication are the circulatory system, nervous system, kidneys, reproductive system, endocrine system, and heart. The mechanism of toxicity occurring in the organ due to absorption plumbum mostly in the depot of the bones and soft tissue, depending on how Pb is exposed and the affinity of tissues. Therefore, any part or organ attacked by Pb toxication will show different effects (Emmanuel S, et al, 2009)

According to Hariono (2005), oral administration of plumbum acetate by 0.5 g/ kg/ day in mice will cause the highest Pb accumulation in soft tissues such as the kidneys, liver, brain, lung, heart, muscle and testis, respectively. On the renal proximal tubular epithelial cells, degeneration, hyperplasia and cariomegali were visible at week 8th, showing the inclusion bodies in the cell nucleus.

When experiencing physiological stress or pathological stimuli, cells could adapt to new conditions and achieve going concern. If the adaptive ability is redundant, cells will undergo excessive injury. Within certain limits the condition is reversible and cell could go back to its original stable state. Severe or persistent stress causes irreversible injury and the affected cells will die (Richard N, Michel MD, Ramzi S, dkk.2003).

Cell degeneration is an event of morphological changes in cells due to injury, which could be reversible and irreversible. Reversible cell injury includes changes in the plasma membrane, mitochondrial changes, dilation of endoplasmic reticulum, and nuclear changes. The morphologic changes could be recognized by light microscope, by the presence of cell swelling and fat degeneration (Richard N, Michel MD, Ramzi S, dkk.2003).

The morphology of irreversible-necrosis cell injury shows sequence of morphologic changes that follow the death of cells in living tissue. Necrosis is macroscopic and histological correlation in cell death occured in the environment of irreversible exogenous injury. The commonest manifestations are coagulated necrosis characterized by cell swelling, cytoplasmic protein denaturation and breakdown of cell organelles. In addition, necrosis also has

the characteristics of membrane protrusion accompanied by loss of membrane integrity, cell lysis and then swell, leaked lysosomes, huddled core, and occurred aggregation (Alberts B, Johnson A, Lewis J, et al, 2000).

Various efforts should be made to deal with lead poisoning, including the use of chelating substance. The chelating material has function to bind Pb by forming complex bonds, which are polar (hydrophilic) and which are removed from the body through the kidneys. According to Suaniti, the success rate of Pb toxicity decrease by EDTA chelating mechanism reached 4.91% and the administration could be done done intravenously. The use of EDTA is therapeutic or curative and has not been maximal. Therefore, it is important to consider preventive measures to prevent the toxicity of Pb. (Suaniti NM, 2007).

Metallothionein protein is rich of sulfhydryl groups, which could be covalently bound to lead in tissues through blocking reaction, which later will enter detoxication process (Santosa B et al, 2013). Metallothionein protein is found in many plant-based ingredients including soy, rice, corn, and beans, either in the roots, stems, leaves, flowers, or fruit. The metallothionein protein content was the highest in rice leaves. (Santosa B, et al, 2015). Rice leaf extract administration should be investigated as an alternative preventive measures degeneration and necrosis of renal tubular epithelial cells.

A research conducted by Santosa et al on levels of metallothionein in vegetable materials consisting of soy, rice, corn, and beans, both in the roots, stems, leaves flowers, and fruit showed that the highest level of the protein was in rice leaves by 1.35 ng. Metallothionein level of 1.39 ng had been demonstrated to decrease significantly the number of basophilic stippling. (Santosa B, et al, 2014). The content of metallothionein protein in rice leaves needs to be studied as an alternative to prevent renal tubular epithelial cell damage in lead-exposed rats, which could be seen from number of cells undergoing degeneration and necrosis.

METHOD

Method used in this research was the *Randomized post test only control-group design*. Maintenance and animal interventions were conducted at the Laboratorium Penelitian dan Pengujian Terpadu (LPPT), Gadjah Mada University, Yogyakarta. Maintenance from selection until the treatment process had been conducted in 8 weeks.

Determination of the number of samples was done by using the formula as follows: $BS = (t - 1)(r - 1) \geq 15$. The number of mice used was 6 for each group (total = 3 treatment and 1 control group), so that the overall number of samples required in this study were 24 eats. One more rat was added to each group as a reserve to anticipate the possibility of dead rats, bringing the total of 28 rats *Rattus norvegicus* to be provided. All mice were male and aged 15 weeks.

To the 1^{st}, 2^{nd} , and 3^{th} treatment group, rice leaf extract was given at a gradual dose of at 0.2, 0.4 and 0.8 ml/ day given through infusion, while the control group was not given the leaf extract of rice. The length of administration period of rice leaf extract was 8 weeks. Pb exposure was given to all groups, both the control and treatment groups, with a dose of 0.5 g/ kg/ bw/ day for 8 weeks at the same time with the supply of rice leaf extract.

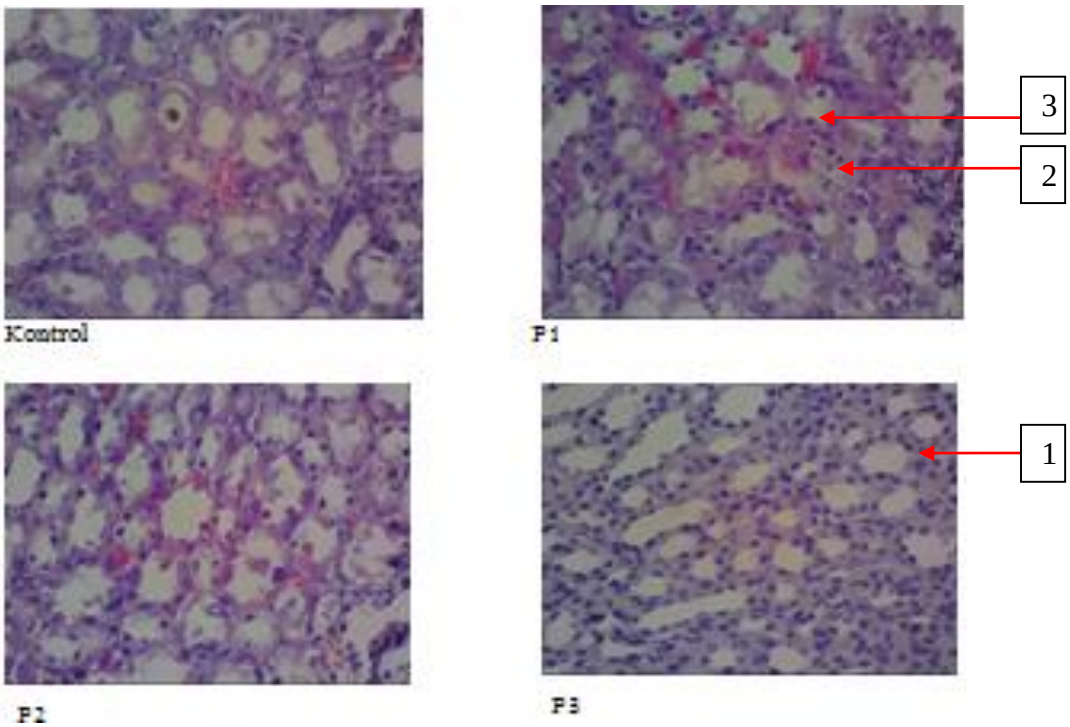
On the last day of the 8-week period, the control and treatment groups were subjected to surgery after being killed by cervical dislocation. To determine the number of cells undergoing degeneration and necrosis, histological preparates were made from the renal organ with hematoxilin eosin staining. Differences in the amount of degeneration and necrosis between control and treatment groups were tested using one-way ANOVA and Spearman rho tests.

The study obtained ethical clearance from the ethics committee of FK Unisula Semarang referred by No.187/VIII/2014/Komisi Bioetik. Results of ethical clearance were notified to the head of the LPPT, Universitas Gadjah Mada, Yogyakarta and were approved for research implementation.

RESULTS

Degeneration dan necrosis of renal epithelial tubuls

The description of the presence of normal, degeneration and necrosis cells in the control and treatment groups (P1, P2, P3) is shown in Figure 1. In the control group, the presence of cell necrosis was seen in almost all of the visual field, and so the cell degeneration was, while the normal cells were very rarely found in the epithelium of the renal tubules. In contrast, in the treatment groups of P1, P2 and P3 respectively, the presence of cell necrosis followed by degeneration of cells decreased, while the presence of normal cells increased.



(a)

FIGURE 1. The presence of normal, degeneration and necrosis cells in the control and treatment groups (P1, P2, P3). Note: 1 normal cells, 2 degeneration, 3 necrosis

TABLE 1. Mean and *p* value of normal cells, degeneration and necrosis of renal tubuls

Group	Normal		Degeneration		Necrosis	
	Rerata	*p*	Mean	*p*	Mean	*p*
Control	13±0,81	0,03	31,2±1,7	0,05	55,7±2,21	0,04
P1	17,5±0,57		28,7±1,25		53,7±1,7	
P2	29,7±0,95	r=0,97	27±1,41	r=-0,92	43,2±0,5	r=-0,93
P3	63,2±1,7		16,7±0,95		21±1,41	

The determination of the p value mean of normal cells, degeneration and necrosis of renal tubules is shown in Table 2 where the average of normal cells increased the highest in the control group to the treatment group, where the lowest was in the control group but the highest in the treatment group (P3). In cells undergoing degeneration and necrosis, the decrease of cell number was in both control and treatment groups, where the highest was in the control group. Yet the lowest was in 3th treatment group (P3).

Based on Kruskal Wallis statistical test on normal, degeneration and necrosis cells, there were significant differences (0.03; 0.05; 0.04) between control and treatment groups (P1, P2, P3). Spearman rho test result showed that there was a strong positive correlation (r = 0.97) in normal cells, a strong negative correlation in cell degeneration and necrosis. (R = -0.92 and r = -0.93), meaning that the higher dose of the extract of rice leaves rice, the higher number of normal cells and the lower the number of cell degeneration and necrosis.

DISCUSSION

Morphology of cell degeneration could be seen by the presence of cytoplasmic swelling, which could be caused by cell injury. Plumbum (Pb) is a substance categorized as heavy metal, which could cause cell injury leading to

degeneration of renal tubular epithelial cells. Effect of rice leaf extract administration given by levels in the treatment groups of *Rattus nurvegicus* rats exposed to Pb affected to total degeneration of tubular cells showed statistically significant differences proving that the content of metallothionein protein contained in rice leaf extract could reduce the impact of Pb exposure. In the degeneration event, cells could be returned to normal cells or progress to necrosis.

Cells tend to defend its immediate neighborhood and intracell within the range of physiological parameters, which are relatively narrow, where cells maintain normal homeostasis. When experiencing physiological stress or pathological stimuli, cells could adapt to new conditions and keep their survival. If excessive adaptation capability is redundant, the cells will undergo injury. Within a certain time limit cell injury is reversible and returned to its original stable condition. When severe stress persists, irreversible injury occurs and cell will die (necrosis) (Richard N, 2003).

The administration of rice leaf extract containing metallothionein, which was given in gradual level in the treatment groups indicated the decline of the number of renal tubular epithelial necrosis and could show statistically significant reduction of necrosis when compared between the control group and all of the treatment groups. The higher doses of rice leaf extract, the lower number of cells that undergo renal tubular necrosis. This possibly because concentration of metallothionein protein was influential binding Pb exposure, which in turn makes it easier to process detoxication. It was found that in the control group, the number of renal tubular necrosis epitelium was higher but then gradually decreased in the 1st, 2nd and 3th treatment. This result is in line with by results of research by Hariono, B (2005) showing that exposure of Pb by 0.5 g/ Kg/ BB/ oral/ day could increase the number of renal tubular epithelial cell necrosis. Research by Haribi R 2007 but using aluminum exposure showed similar renal tubular epithelial necrosis phenomenon.

CONCLUSION

Rice leaf extract is capable as the prevention of kidney damage in *Rattus nurvegicus* rats exposed by Pb.

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